ance of collagen in those cartilage tissue complex. Homologously, compared with none herbal intervention group, the expression of collagen II in cartilage-liked tissue in HBP-A intervention group was also improved in PCR assay with 0.80 [95%CI = 0.67,0.93] vs 0.49 [95%CI = 0.45,0.53] ($P<0.05$). However, the mRNA expression of aggrecan was failed to demonstrate difference with 0.59 [95%CI = 0.40,0.78] vs 0.50 [95%CI = 0.28,0.71] ($P>0.05$).
Conclusions: In a summary,cartilage like tissue could be found after the injection of alginate hydrogel embedded with chondrocytes and HBP-A. The study documented that the potential pharmacological target of Chinese herbal extract HBP-A in the application of cartilage tissue engineering may be concerned with the inhibition of catabolic enzymes MMP-3 and ADAMTS-5, and increasing of collagen II expression,and further study should be explored.
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493

THE CHARACTERISTIC OF MESENHYMAL STROMAL CELLS CULTURE DERIVED FROM KNEE FAT PAD

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Purpose: As the hyaline cartilage has a limited reparative potential the cells culturing technologies became attractive method for restoration of full-thickness cartilage defects. We evaluated the differentiation potential of multipotent mesenhymal stromal cells (MMSC) culture derived from knee fat pad.
Methods: During knee arthroscopy in 3 patients with full-thickness traumatic cartilage lesions 1 cm^3 of fat pad was resected, subjected to fermental dissociation and than culturing for 3 passages in order to get 6 millions cells. The characteristics of cells culture were confirmed by “Minimal criteria for defining multipotent mesenhymal stromal cells” according to “The International Society for Cellular Therapy position statement” and some additional investigations.
Results: We found that cells culture from fat pad adhere to the surface of culturing plastic, has the similar surface markers as MMSC: CD73 (96,3-100%), CD44 (92,1-99,4%), CD105 (94,9-100%), CD90 (94,9-99%); in minimal extent expresses hemopaethic markers CD34 (0,05-3%), CD45 (0,01-22%), CD71 (-), CD106 (2,03-13,2%), CD11b (3,7%), CD10 (2,8-4%) and didn’t express CD133. This cells culture has wide differentiating potential during the induction of differentiation in orthodoxal directions - osteogenic, chondrogenic and adipocitogenic. Osteogenic differentiation was confirmed by high synthetic activity of alkaline phosphatase, osteocalcinum expression and ability to form mineral precipitates. Chondrogenic differentiation was confirmed by forming of chondrocytal spheroids and gystochemically by identification of glucosaminoglycans and collagen type II. Adypogenic differentiation was confirmed by accumulation in cytoplasm a lot of adiposal drops that positive stained by sudan 3.
Conclusions: Thereby the multipotent mesenhymal stromal cells culture derived from knee fat pad has good potential to be used for restoration of full-thickness cartilage defects.

494

EXTRACELLULAR MATRIX DEPOSITION BY CHONDROGENICALLY DIFFERENTIATING HUMAN MESENCHYMAL STEM CELLS IS ENHANCED BY MACROMOLECULAR CROWDING

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Purpose: Mesenchymal stem cells (hMSCs) are a potential source of cells for cartilage tissue engineering. During chondrogenic differentiation of hMSCs, the accumulation of macromolecular extracellular matrix (ECM) components, specifically collagens and glycosaminoglycans (GAG), is critical for the generation of a functional, mechanically competent tissue. The macromolecular ECM components are synthesized as monomers or individual molecules, and their assembly into a cohesive matrix depends largely on their local concentration. In a cell-culture environment, the bulk of the ECM components synthesized by the cells is typically lost by diffusion into the cell culture medium. Others have reported that collagen incorporation by fibroblast cultures is enhanced by the addition of exogenous macromolecules to the medium. Macromolecular crowding has been proposed as the underlying mechanism for this phenomenon; the exogenous macromolecules occupy a certain volume and consequently limit the volume available to other molecules, increasing their local concentration. In this study, we evaluated the effects of several macromolecular crowders on chondrogenically differentiating hMSCs in an aggregate culture system.
Methods: hMSCs were culture-expanded and then placed in chondrogenic aggregate culture in 96-well plates (250×10^3 cells/well). Polystyrene (PS, 300kDa), dextran sulfate (DS, 500 kDa), and hyaluronic acid (HA, 350 kDa) were added to the chondrogenic differentiation medium at 0.1 mg/ml. We tested three different exposure durations (3, 5, or 7 days after aggregate formation) on two different types of aggregate cultures using multiple cell preparations. The first type was pelleted as undifferentiated MSCs on day 0 and maintained as aggregates for the duration of the experiment. The second type was pre-differentiated in aggregate culture for 7 days, before being dissociated and re-pelleted as pre-differentiated cells on day 7. After 14 or 21 days, the aggregates were harvested for histological and quantitative biochemical analyses.
Results: The addition of crowding macromolecules had different effects depending on the differentiation state of the cells. Crowding had little effect on the undifferentiated MSCs or even decreased ECM deposition slightly. Treatment of the pre-differentiated MSCs resulted in up to a 300% increase in GAG accumulation, with similarly increased collagen deposition, and minimal changes to the DNA content of the aggregates. These aggregates were larger, proportionally to the GAG content. Although all three macromolecules caused an increase in ECM incorporation, PS had the largest effect, followed by DS and HA. The longer exposure times had consistently larger effects than the shorter ones.
Conclusions: Macromolecular crowding increases and accelerates the amount of collagen and GAG deposited by differentiating hMSCs. The use of crowding macromolecules can, therefore, potentially enhance tissue-engineering applications, and our long range goals therefore include extending these studies to large-scale tissue engineered cartilage constructs. What determines the differences in effectiveness between polystyrene and the other macromolecules, and what the optimal dosing regimen is, remains to be determined. It also seems likely that these mechanisms underlie to some degree the effects of viscosupplementation in vivo.